STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY

PAUL BERG
*Willson Professor of Biochemistry*

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Dr. William Gartland
Executive Secretary
NIH Committee on Recombinant DNA
Molecule Biohazards
NIH
Bethesda, Maryland 20014

Dear Dr. Gartland,

I am writing to you about a recombinant DNA experiment, which, according to the Dec. 22, 1978 Revised Guidelines, requires case by case approval. I assume that proposals for such approval need to be reviewed in your office or by some ad hoc review group and approved by the Director. Would you advise me as to how long that review and approval is likely to take?

Herpes simplex virus (Type I)(HSV 1) carries a structural gene for thymidine kinase (TK) in its genome and cells infected with the virus produce a HSV-1 specific TK, that is, a TK readily distinguishable from cellular TK. Richard Axel and his colleagues have shown that the structural gene for HSV TK is contained within a 3.4 kb fragment (produced by Bam Hl endonuclease digestion) and that $TK^-$ mouse L cells can be transformed to a $TK^+$ phenotype by exposure to the Bam Hl DNA fragment. The same Bam Hl DNA fragment has been cloned in a bacterial EK2 system (PBR322 and X1776) in several different laboratories (e.g. Richard Axel's, George Stark's and very likely several others). The availability of a cloned mammalian TK gene makes feasible several interesting and important experiments. We plan to insert the HSV-TK DNA segment into the defective SV40 genome to obtain a vector capable of producing TK following infection or transformation of mammalian cells, particularly $TK^-$ derivatives. Many experiments not now feasible or practical could be undertaken if we had a SV40 vector carrying a selective marker.

The HSV-TK mRNA is estimated to have a molecular size of about 1.4 kb and the Bam Hl fragment that contains its coding sequence is 3.4 kb. The precise boundaries of the TK gene are as yet unknown although preliminary results from William Summers' lab suggest it may be at one end of the 3.4 kb segment.

Several approaches could be taken to clone the TK gene:

1. Insertion of the cloned 3.4 kb segment into an appropriate defective SV40 vector (3-4 kb in size) to yield recombinant DNAs that are too large to be encapsidated into virions i.e. they can not produce infectious progeny. Such SV40-TK recombinants would be introduced into $TK^-$ permissive and non-

permissive cells to select for $TK^+$ derivatives.

2. TK cDNA homologous to the 1.4 kb TK mRNA could be cloned using P2-EK2 containment; this purified cDNA would then be introduced into a suitable SV40 vector to yield a recombinant that could express TK.

3. The 3.4 kb Bam Hl fragment could be restricted by a variety of restriction enzymes to obtain the smallest DNA segment capable of inducing TK when transfected into $TK^-$ mouse L cells. The segment, if small enough to exclude the possibility of additional genes (e.g. it could be larger than 1.4 kb if introns were present or if a promoter sequence proximal to the coding segment was part of the fragment) would be introduced into appropriate SV40 vectors for vegetative propagation or cellular transformation.

The latter two approaches, but not the first, can be undertaken under present Guidelines; the the first and the final phases of the latter two require approval from NIH before they can be undertaken. In my own view approach #1 is without significant risk since no virus can be produced and containment of cultured cells is acknowledged to be a relatively trivial matter. The latter two approaches yield defective SV40-TK recombinants that pose no ascertainable risk in as much as the TK is innocuous. In short I can not conceive of credible risks in any of these experiments and, therefore, request that you try to expedite their approval. I understand that Dr. David Baltimore has also put before RAC a proposal to clone HSV-TK with Moloney retrovirus vectors. Hopefully both of these proposals can be reviewed at the same time.

Sincerely yours,

Paul Berg

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